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ADHESION AND BIOCIDES INACTIVATION OF *SALMONELLA* ON STAINLESS STEEL AND POLYETHYLENE

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ABSTRACT

The adhesion of *Salmonella* (*S.*) strains to stainless steel and polyethylene and their inactivation by biocides used in food industry was investigated. Coupons of stainless steel and polyethylene were immersed in bacterial suspensions of *S*. Enteritidis, *S*. Typhimurium, and *S*. Bredeney during 15, 30, and 60 minutes, and submitted to different concentrations of peracetic acid (PAA), sodium hypochlorite (NaOCl), and quaternary ammonium (Quat) sanitizers. Hydrophobicity of the surfaces was evaluated by contact angle measurements using the sessile drop method and bacterial adhesion was accompanied through bacterial counts and scanning electron microscopy (SEM). Results indicated that the three serovars of *Salmonella* presented similar adhesion to both materials (5.0 to 6.5 log cfu cm^{-2}). The time of exposure did not influence the counts of adhered cells on both surfaces, however SEM revealed larger clusters of *S.* Enteritidis on both materials, not found for the other serovars. *S.* Enteritidis presented lower sessile drop angle on polyethylene, indicating hydrophilic properties of this material. The biocides were not able to inactivate all the microorganisms adhered on both surfaces. At least 1 log cfu cm^{-2} of all serovars tested remained viable after the exposure to different biocide concentrations. In general, higher counts of survivors were observed on polyethylene disinfected with different concentrations of biocides. *S.* Bredeney e *S.* Typhimurium were more resistant than *S*. Enteritidis to PAA, whilst *S*. Enteritidis presented smaller reduction rates to NaOCl. This last biocide was able to reduce *Salmonella* counts in approximately 3.0 to 4.0 log cm^{-2}. When adhered to polyethylene, the serovars *S*. Typhimurium and *S*. Enteritidis were more resistant to Quat than *S*. Bredeney in all concentrations tested, and the numbers of *S*. Enteritidis remained almost unaltered. On stainless steel disinfected by Quat, *S*. Bredeney presented higher numbers of survivors.

Key words: *Salmonella*; adhesion; biocides; stainless steel; polyethylene.

INTRODUCTION

Salmonellosis is an important public health problem worldwide and the control of *Salmonella* in food chain remains difficult. In Brazil, *Salmonella* has been identified as the main cause of foodborne diseases investigated by regulatory bodies, and *S*. Enteritidis has been identified as the most frequent serovar involved in foodborne outbreaks (20, 21). In Rio Grande do Sul (RS), Southernmost state of Brazil, a specific strain of *S*. Enteritidis (SE86) has been the causative agent of more than 90% of the salmonellosis investigated in the last years (8, 21, 22). The factors that make this strain an important

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foodborne pathogen are still unclear, and could be related to its adhesion capacity or resistance to usual sanitizers.

Bacterial adhesion is influenced by the type of surface and its topography, considering that abrasive surfaces are more susceptible to dirt accumulation and are more difficult to clean than smoother surfaces (7, 9, 17). Other factors such as hydrophobicity, chemical composition and the presence of proteins on the surfaces can also result in different levels of bacterial adherence (4, 11, 27).

Stainless steel and polyethylene have been largely employed in equipments and utensils for food production, due to their resistance, durability, and easiness in the cleaning process and disinfection. However, the sanitation procedures applied on such surfaces could be not completely effective, since effectiveness depends on the type of adhered microorganisms and the properties of these surfaces (2). The choice of adequate sanitizers is very important, and must be preceded of a detailed analysis, such as ~~its~~ the official authorization of use, toxicity, corrosive action, residual effect in the food, environmental impact and cost (26). Peracetic acid, sodium hypochlorite and quaternary ammonium are some chemical agents that fulfill these characteristics (18).

In Brazil, sodium hypochlorite (NaOCl) has been widely used in home and food establishments, due to its efficiency against many microorganisms and accessible cost (24). Peracetic acid (PAA) has also been frequently used, since it is very efficient to remove biofilms and causes lower environmental impact (10, 17, 26). The use of quaternary ammonium (Quat) compounds is also very common, due to their antimicrobial properties, low toxicity and effectiveness for surfaces disinfection (18).

The aim of this work was to evaluate the adhesion of *Salmonella* strains, involved or not with salmonellosis, to stainless steel and polyethylene and also to investigate the ability of NaOCl , PAA and Quat to disinfect these materials.

MATERIALS AND METHODS

Bacterial strains

Strains of *Salmonella enterica* of three different serovars were used in this study. *S.* Enteritidis strain (SE86) was isolated from cabbage involved in a salmonellosis outbreak occurred in Rio Grande do Sul (RS), in 1999. This microorganism presents the same genotypic pattern of *S.* Enteritidis strains involved in more than 90% of the outbreaks of salmonellosis occurred in the period between 1999 and 2002, in RS (8, 21). *S.* Typhimurium and *S.* Bredeney strains were chosen because they were isolated in RS State in 1999, from a pig fecal sample and from a fermented meat sample, respectively, and these *Salmonella* serovars rarely were involved in foodborne diseases in RS in the last years (21). The strains were stored at – 18ºC in 50% (v/v) glycerol. They were activated by transferring 20 µl of stock culture to 3 ml of Brain Heart Infusion (BHI) (Biobras, Belo Horizonte, Brazil). Working cultures were kept at 4º C on Nutrient Agar (NA) (Merck, Darmstadt, Germany) plates, and before use they were transferred to Nutrient Broth (NB) (Synth, São Paulo, Brazil) and incubated for 24 h at 37º C.

Preparation of stainless steel and polyethylene coupons

Stainless steel AISI 316 (0.1 cm thick) (Metalbras, Porto Alegre, Brazil) and polyethylene (0.7 cm thick) (Sanremo, Esteio, Brazil) coupons of 2 x 2 cm were prepared. Prior to adhesion tests, coupons were degreased with a neutral detergent (3%, v/v) for one hour, rinsed with 70% (v/v) ethanol, and then washed with distillated water. The coupons were then dried at 60° C for two hours and autoclaved at 121° C, for 15 min in sealed tubes (26).

Coupons contamination and evaluation of *Salmonella* adherence

The coupons were immersed in 10 ml of NB containing approximately 8 log cfu ml^{-1} of each strain separately. Three coupons of stainless steel and three coupons of polyethylene were immersed in the cultures for 15, 30, and 60 min, without shaking, at room temperature (14). These times were chosen aiming to simulate the short time of contact of food with surfaces during food preparation. After that, the coupons were washed with PBS (phosphate buffer saline; pH 7.2) to remove

the poorly adhered cells. The stainless steel coupons were immersed in 10 ml of PBS, while the polyethylene coupons were immersed in 15 ml of PBS before sonication process. Each coupon was submitted to sonication in a bath sonicator (UNIQUE USC 700) with frequency of 40 KHz, for 2 periods of 10 min, for the release of adhered cells. During sonication, the temperature of PBS was monitored with a Thermometer (AKSO MULTI-Thermometer AKTD 3429) and it not exceeded 40^{o} C, avoiding thermal injury to *Salmonella* cells.

PBS containing each sonicated coupons were submitted to decimal dilutions and 20 µl of each dilution were plated in NA, as described by Milles and Misra (19). The plates were incubated for 18 hours at 37° C and the numbers of cfu cm^{-2} were determined. All counts were made in triplicate and each experiment was repeated twice.

Hydrophobicity evaluation

Hydrophobicity was evaluated by the sessile drop method described by Locatelli *et al.* (15), measuring the contact angles of the testing materials in the stainless steel and polyethylene coupons. Drops of 20 µl of the cultures of each one of the three *Salmonella* serovars in BHI were added to the surface of cleaned and disinfected coupons and the contact angles were compared to that obtained using 20 µl of distillated water. The drops were registered by a digital camera (Sony® Cyber-Shot 5MP model F707) in the photodocumentation section of the Hospital de Clínicas of Porto Alegre (HCPA) and the images were analysed in a personal computer. The contact angle was measured through the inclination of the line formed between the contact base and the drop height, larger angles meaning higher hidrophobicity (28). All measurements were performed at room temperature.

Scanning electron microscopy

Scanning electron microscopy was carried out to evaluate bacterial adhesion to stainless steel and polyethylene. For these tests, bacterial cultures with 8 log cfu ml^{-1} remained in contact with the polyethylene for 15 min and with the stainless steel for 30 min. The coupons were prepared as follows: coupons with adhered cells were washed three times for 30 min with 0.2 mmol l^{-1} phosphate buffer and distillated water (1:1), fixed with 12% (v/v) glutaraldehyde for 7 days, and washed again with 0.2 mmol l^{-1} phosphate buffer. The coupons were dehydrated with acetone in increasing concentrations of 30 to 100 %, with a pause of 10 and 20 minutes. After drying in room temperature, the coupons were submitted to critical point drying with liquid CO_2, in the Balzers CPD030 equipment (Balzers Union Ltd, Balzers, Lichtenstein). The coupons were covered with gold (metallization) in Balzers SCD050 equipment (Balzers Union Ltd, Balzers, Lichtenstein), and observed in a Jeol JSM-6060 scanning electron microscope (Jeol, Tokyo, Japan).

Resistance of adhered cells to sanitizers

The stainless steel and polyethylene coupons were immersed for 15 min in bacterial suspensions of each *Salmonella* serovar. After that, the coupons were transferred to recipients containing 10 ml of the sanitizers in the following concentrations: 150, 300, 450, 750, and 1500 mg kg^{-1} PAA; 40, 120, 200, 400, and 800 mg kg^{-1} NaOCl; 400, 600, 2000, and 4000 mg kg^{-1} Quat. To each sanitizer tube, 1 ml of bovine serum albumin (10 mg ml^{-1}) was added. Each coupon was separately immersed for 10 min in each sanitizer concentration, and then immersed again in neutralizing solutions for 30 seconds (12). The neutralizing solutions were 0.6% (w/v) sodium thiosulfate to NaOCl and PAA, and 0.5% (v/v) Tween 80 to Quat (12, 13). The samples were then transferred to PBS and immediately sonicated for two periods of 10 min. The coupons used as control were treated with distillated water instead of sanitizers. Samples were submitted to decimal dilutions, and 20 µl of each dilution were plated in NA, for determination of viable cell counts (19).

Statistical analysis

Data were subjected to variance analysis (ANOVA) and Tukey's test to detect significant differences Differences were considered significant when $P<0.05$.

RESULTS

Evaluation of the adhesion capability

The three serovars of *Salmonella* presented similar adhered cell counts, ranging from 5.27 to 5.89 log cfu cm^{-2}, on stainless steel, and from 4.8 to 6.45 log cfu cm^{-2}, on polyethylene (Table 1). The time of exposure did not influence (P<0.05) the counts of adhered cells on both surfaces. *S*. Bredeney adhered better to polyethylene, whereas *S*. Typhimurium to stainless steel (P<0.05). *S*. Enteritidis revealed similar adhesion levels to both materials. Scanning Electron Microscopy revealed that polyethylene presented a much deeper and irregular surface than the stainless steel (Figure 1 A and B). In general, microorganisms presented similar distribution on the materials, except by the fact of *S*. Enteritidis was able to form clusters of cells on stainless steel (Figure 1 C) and on polyethylene (Figure 1 D), what was not observed by the other serovars (result not shown).

Table 1. Adhesion of *Salmonella* serovars on stainless steel and polyethylene coupons.*

CONTACT TIME (MIN)	*S*. Bredeney	*S*. Typhimurium	*S*. Enteritidis
Stainless steel			
15	5.63 ± 0.34	5.68 ± 0.10	5.27 ± 0.17
30	5.70 ± 0.55	5.66 ± 0.54	5.53 ± 0.15
60	5.75 ± 0.51	5.89 ± 0.15	5.43 ± 0.20
Polyethylene			
15	6.22 ± 0.15	5.30 ± 0.01	4.80 ± 0.41
30	6.39 ± 0.08	5.46 ± 0.15	5.17 ± 0.12
60	6.45 ± 0.01	5.64 ± 0.04	5.19 ± 0.21

* Values (log_{10} cfu cm^{-2}) are the means ± standard deviations of three independent experiments.

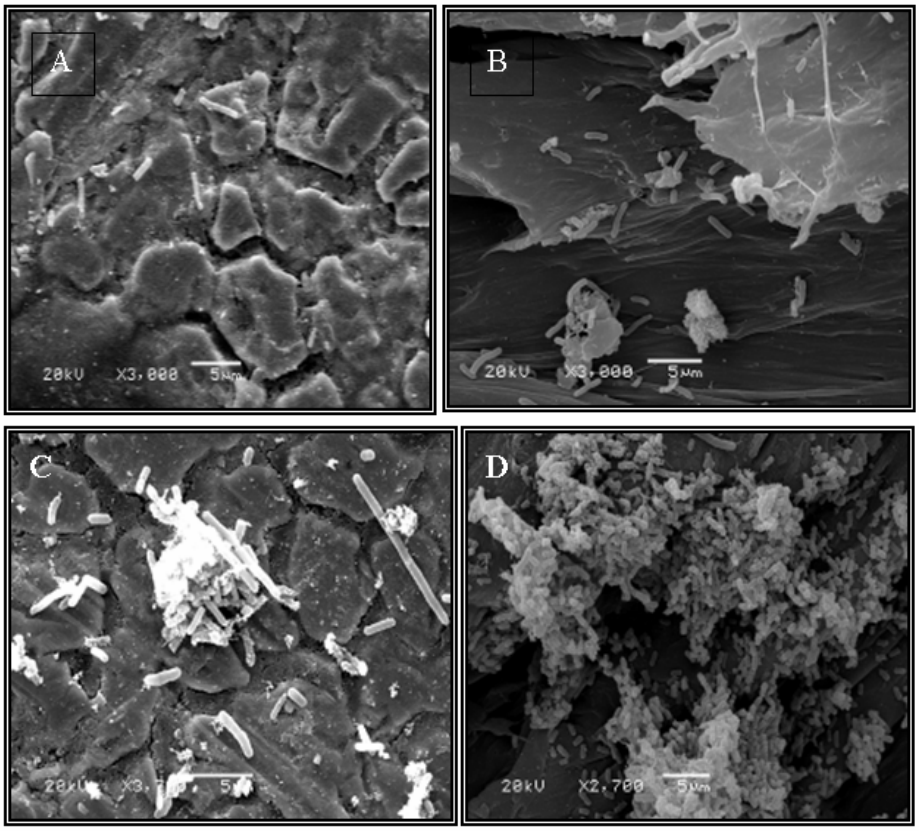

Figure 1. Scanning electron microscopy (SEM) showing the surfaces of stainless steel (A) and polyethylene (B) coupons, and the clusters of adhered cells of *S*. Enteritidis on stainless steel (C) and on polyethylene (D).

Hydrophobicity evaluation

The drop contact angle measurements on polyethylene surfaces were higher than those observed on stainless steel (Table 2 and Figure 5), indicating that polyethylene was more hydrophobic than stainless steel.

An expressive reduction of the sessile drop angle on polyethylene could be observed when those obtained with no inoculated BHI (60.7º ± 2.5) were compared with BHI containing *S.* Enteritidis cells (44° ± 2.8). These results suggest that *S.* Enteritidis cells present hydrophilic properties. Suspensions of *S.* Enteritidis showed similar angles on both stainless steel and polyethylene, while *S.* Typhimurium and *S.* Bredeney showed higher drop angles on polyethylene (Table 2).

The three *Salmonella* serovars presented similar drop angle measurements on stainless steel. In contrast, the same result was not observed for polyethylene, once the drop angle measurement for *S.* Enteritidis culture was statistically different from the other two serovars ($P < 0.05$).

Table 2. Measurement of sessile drop angle in stainless steel and polyethylene surfaces of *S.* Typhimurium, *S.* Enteritidis and *S.* Bredeney cultures.*

	DISTILLED WATER	BHI†	BHI+ST	BHI+SE	BHI+SB
Stainless steel	44.5° ± 0.7	50.5° ± 0.7	45° ± 1.4	42°± 1.4	44°±1.4
Polyethylene	52.2°± 1.8	60.7º ±2.4	51° ± 0.0	44° ± 2.8	52°±1.4

* Values are the means ± standard deviations of three independent determinations.
† BHI, Brain Heart Infusion broth; ST, *S.* Typhimurium; SE, *S.* Enteritidis; SB, *S.* Bredeney.

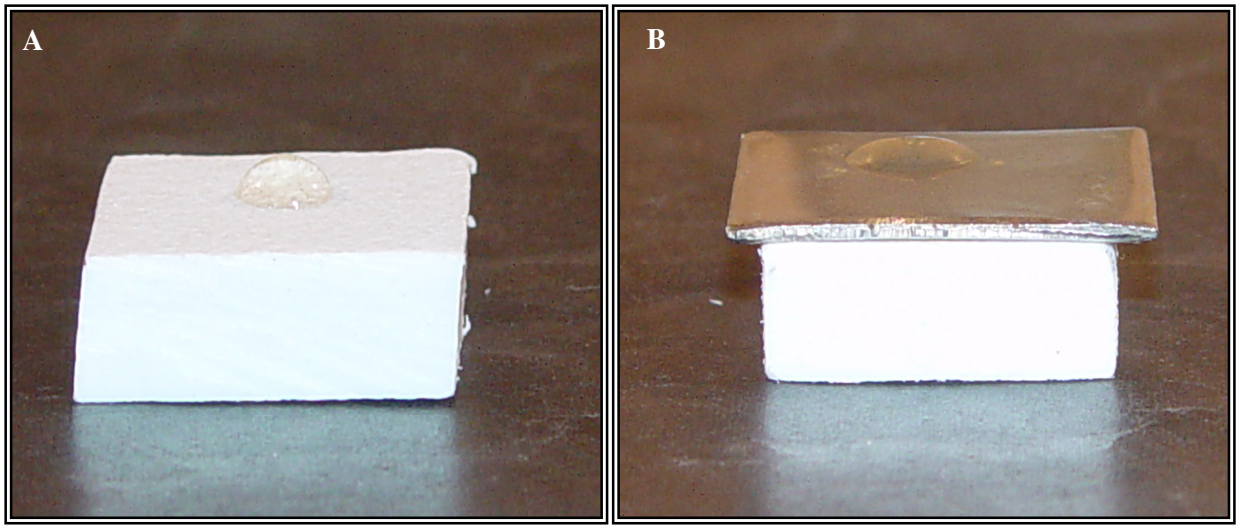

Figure 5. Coupons of polyethylene (A) and stainless steel (B) with a drop of Brain Heart Infusion broth, used for hydrophobicity measureme
the sessile drop method.

Resistance of adhered cells to sanitizers

The survival of the three *Salmonella* serovars to PAA is shown in Fig. 2 and Table 3. *S*. Bredeney, *S.* Typhimurium, and *S*. Enteritidis were the most resistant to disinfection when adhered to polyethylene, under all concentrations of PAA tested (Fig. 2A, 2B, and 2C). The use of PAA at 750 and 1500 mg kg^{-1} caused a significant reduction in the counts of *S.* Bredeney and *S.* Enteritidis on stainless steel when compared to polyethylene (P<0.05). The reductions rates varied approximately of 2.4 to 3.3 log cm^{-2}, on polyethylene, and of 2.5 to 4.0 log cm^{-2}, on stainless steel.

The survival of the three *Salmonella* serovars adhered to polyethylene and stainless steel after treatment with different concentrations of NaOCl is illustrated in Fig. 3 and Table 3. *S.* Bredeney (Fig. 3A) and *S*. Typhimurium (Fig. 3B) showed similar resistance to NaOCl when adhered to polyethylene or stainless steel, under all NaOCl concentrations tested. However, the final counts of *S.* Bredeney were higher than the final counts of *S*. Typhimurium. *S*. Enteritidis (Fig. 3C) was more resistant to the disinfection of NaOCl (800 mg kg^{-1}) on polyethylene, once-reduction rates were smaller (2.7 log cm^{-2}) than the reduction rates of the other serovars (3.1 log cm^{-2} for *S*. Bredeney and 3.7 log cm^{-2} for *S.* Typhimurium).

The survival of the three *Salmonella* serovars after the treatment with different concentrations of Quat is presented in Fig. 4 and Table 3. Overall, the microorganisms presented higher numbers of survivors on polyethylene treated with biocides than on stainless steel. When adhered to polyethylene, the serovars *S*. Typhimurium (Fig. 4B) and *S*. Enteritidis (Fig. 4C) were more resistant to Quat (P<0.05) than *S*. Bredeney (Fig 4A), in all concentrations tested. *S*. Enteritidis showed the smaller reduction rate (1.22 log log cm^{-2}) after the disinfection of polyethylene with Quat compared to *S*. Typhimurium (2.52 log cm^{-2}) and *S*. Bredeney (4.12 log cm^{-2}). On stainless steel, *S*. Typhimurium was more resistant to Quat than the other serovars, once the maximum reduction observed was 4.44 log cm^{-2}, whereas *S.* Bredeney presented 5.21 log cm^{-2} reduction and *S*. Enteritidis 6.24 log cm^{-2} reduction.

When the three *Salmonella* serovars were exposed to PAA concentrations of 1500 mg kg^{-1} and 750 mg kg^{-1} in suspension tests (with planktonic cells), this biocide was able to completely inactivate all microorganisms within 5 minutes. Indeed, Quat at 4000 mg kg^{-1} and 2000 mg kg^{-1} and NaOCl at 800 mg kg^{-1} were able to inactivate all *Salmonella* serovars in few minutes. However, *S*. Enteritidis was the only serovar that resisted to 200 mg kg^{-1} of NaOCl for 15 minutes of exposure in suspension tests (results not shown).

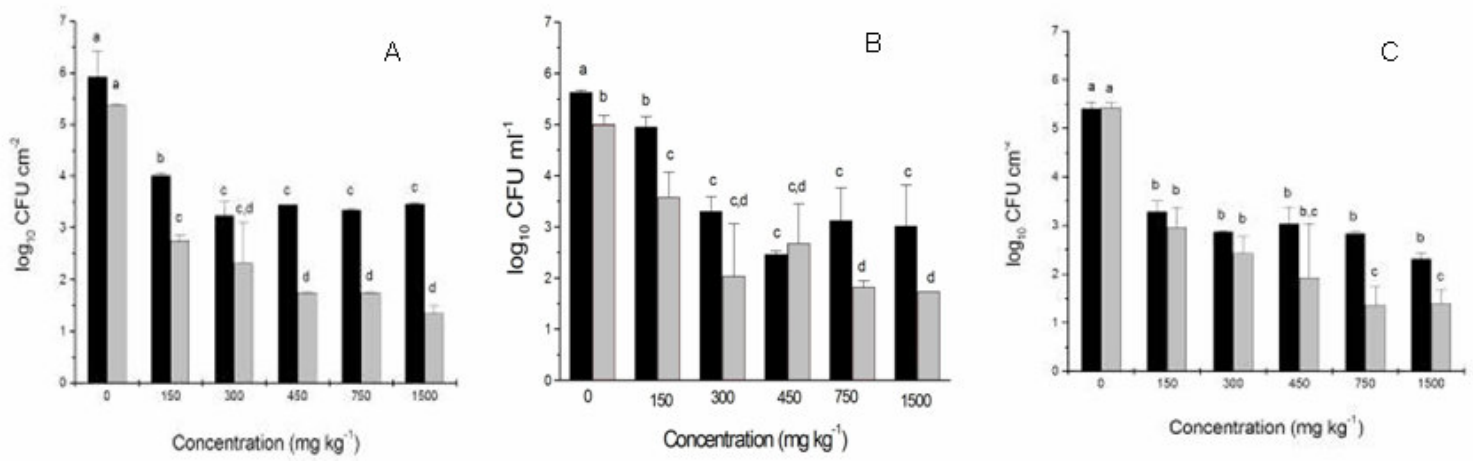

Figure 2. Survival of *S.* Bredeney (A), *S*. Typhimurium (B) and *S*. Enteritidis (C) adhered to stainless steel (grey columns) and polyethy (black columns) coupons after treatment with peracetic acid for 10 min.

Table 3. Number of survivors (log CFU/cm^2) of three *Salmonella* serovars adhered to stainless steel (ss) and polyethylene (pol) coupons after 10 minutes of treatment with different concentrations of peracetic acid (PAA), sodium hypochlorite (NaOCl), and quaternary ammonium (Quat) biocides.

PERACETIC ACID (PAA)						
Concentrations mg Kg^{-1}	***S*. Bredeney (log CFU/cm^2)**		***S*. Typhimurium (log CFU/cm^2)**		***S*. Enteritidis (log CFU/cm^2)**	
	ss	**pol**	**ss**	**pol**	**ss**	**poly**
Control	5.36 ± 0.02	5.91 ± 0.49	5.01 ± 0.17	5.63 ± 0.04	5.42 ± 0.10	5.40 ± 0.13
150	2.75 ± 0.00	4.01 ± 0.05	3.58 ± 0.49	4.96 ± 0.20	2.96 ± 0.41	3.28 ± 0.21
300	2.31 ± 0.79	3.23 ± 0.26	2.04 ± 0.42	3.31 ± 0.28	2.42 ± 0.35	2.86 ± 0.02
450	1.74 ± 0.00	3.44 ± 0.004	2.68 ± 0.77	2.47 ± 0.06	1.92 ± 1.10	3.02 ± 0.35
750	1.74 ± 0.00	3.34 ± 0.02	1.83 ± 0.12	3.13 ± 0.63	1.35 ± 0.39	2.83 ± 0.03
1500	1.33 ± 0.15	3.46 ± 0.01	1.74 ± 0.00	3.02 ± 0.80	1.38 ± 0.29	2.31 ± 0.11
SODIUM HYPOCHLORITE (NAOCL)						
Concentrations mg Kg^{-1}	***S*. Bredeney (log CFU/cm^2)**		***S*. Typhimurium (log CFU/cm^2)**		***S*. Enteritidis (log CFU/cm^2)**	
	ss	**pol**	**ss**	**pol**	**ss**	**poly**
Control	5.73 ± 0.23	5.70 ± 0.35	5.55 ± 0.01	5.56 ± 0.06	5.54 ± 0.36	5.36 ± 0.19
20	5.56 ± 0.03	5.33 ± 0.07	5.06 ± 0.14	5.43 ± 0.05	5.35 ± 0.24	5.18 ± 0.35
40	4.88 ± 0.83	4.79 ± 0.63	4.72 ± 0.02	5.00 ± 0.03	5.02 ± 0.09	4.34 ± 0.09
120	3.52 ± 0.02	3.86 ± 0.60	2.93 ± 0.08	4.78 ± 0.31	3.26 ± 0.39	4.26 ± 0.11
200	3.42 ± 0.05	3.83 ± 0.74	2.55 ± 0.28	3.81 ± 0.47	3.68 ± 0.06	4.18 ± 0.06
400	2.37 ± 0.08	3.12 ± 0.09	2.72 ± 0.00	2.80 ± 0.01	2.68 ± 0.11	3.93 ± 0.03
800	2.54 ± 0.04	2.59 ± 0.61	1.38 ± 0.21	1.88 ± 0.25	1.03 ± 0.00	2.62 ± 0.08
QUATERNARY AMMONIUM (QUAT)						
Concentrations mg Kg^{-1}	***S*. Bredeney (log CFU/cm^2)**		***S*. Typhimurium (log CFU/cm^2)**		***S*. Enteritidis (log CFU/cm^2)**	
	ss	**pol**	**ss**	**pol**	**ss**	**poly**
Control	5.73 ± 0.23	5.70 ± 0.35	5.34 ± 0.09	5.12 ± 0.14	6.24 ± 0.98	5.29 ± 0.60
200	4.88 ± 0.83	4.79 ± 0.63	1.59 ± 2.25	4.14 ± 1.18	2.28 ± 0.27	5.06 ± 0.25
400	3.52 ± 0.02	3.86 ± 0.60	1.66 ± 2.34	4.11 ± 1.81	1.74 ± 0.65	5.12 ± 0.19
600	3.42 ± 0.05	3.83 ± 0.74	2.86 ±0.98	3.67 ± 1.01	1.59 ± 0.21	5.19 ± 0.18
2000	2.37 ± 0.08	3.12 ± 0.09	0.90 ±1.28	2.83 ± 0.21	0.87 ± 1.23	4.89 ± 0.31
4000	0.52 ± 0.73	1.58 ± 0.62	0.92 ±1.31	2.60 ± 0.14	ND	4.07 ± 1.36

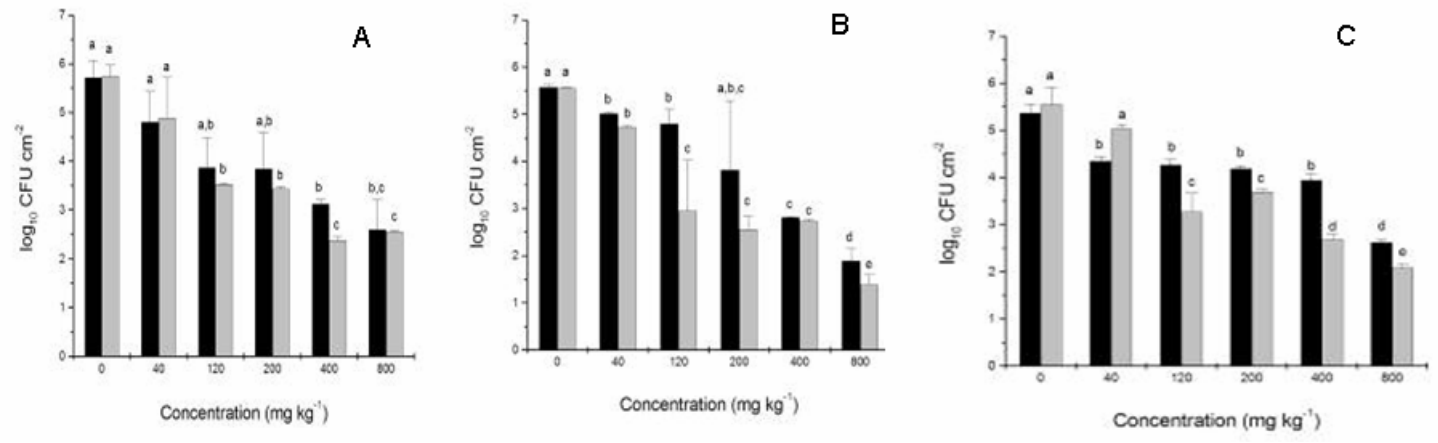

Figure 3. Survival of *S.* Bredeney (A), *S*. Typhimurium (B) and *S*. Enteritidis (C) adhered to stainless steel (grey columns) and polyethylene (black columns) coupons after treatment with sodium hypochlorite for 10 min.

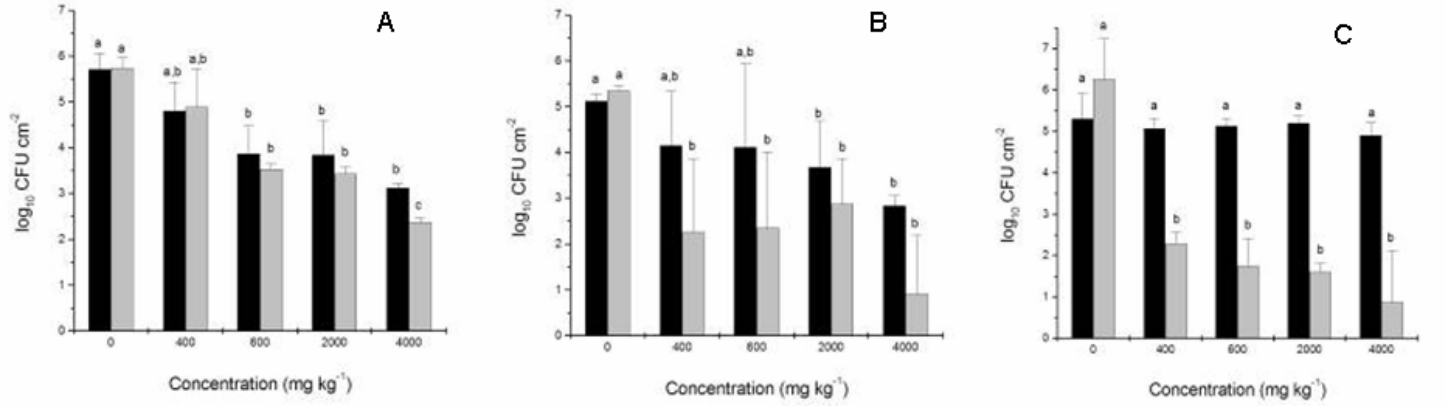

Figure 4. Survival of *S.* Bredeney (A), *S*. Typhimurium (B) and *S*. Enteritidis (C) adhered to stainless steel (grey columns) and polyethylene (black columns) coupons after treatment with Quat for 10 min.

DISCUSSION

Salmonella serovars tested in this study showed an adhesion capacity between 5.0 and 6.0 log cfu cm^{-2} on stainless steel, and between 5.0 and 6.45 log cfu cm^{-2} on polyethylene. These results are in agreement with Joseph *et al.* (12) who reported that *Salmonella* sp. were able to form biofilms of about 5 log cfu cm^{-2} on stainless steel and 7 log cfu cm^{-2} on polyethylene. Similar results were also observed by Oliveira *et al.* (25) studying the adhesion of different strains of *S.* Enteritidis to stainless steel SS 304. In our study the tested serovars presented no significant differences in adhesion levels when exposed by different times on stainless steel or polyethylene, suggesting that the exposure periods did not influence the final amount of adhered cells. Hood and Zotolla (11) quantified biofilms formed by many bacteria, including *S.* Typhimurium on stainless steel for contact periods of 1 to 72 hours, and found adhesion levels around 5 log cfu cm^{-2}. Those results suggested that the microorganism adherence did not increase after one hour of contact.

Stainless steel and polyethylene are widely used in food processing equipments, and are able to be colonized by *Salmonella* and other microorganisms. Adhered bacteria to equipment surfaces can have the potential to act as sources of microbial contamination, which may compromise food quality and represent a significant health hazard (1, 5, 23). According to our results, even short periods of contact, such as 15 min, may allow a considerable number of *Salmonella* cells to adhere, demonstrating a potential risk of cross contamination during food handling.

In the present study, polyethylene was shown to be more hydrophobic than stainless steel, once the drops in contact with polyethylene surface presented higher inclination angles. Hydrophobic materials are reported as surfaces that provide a greater bacterial adherence (6**, 15**). The greater hydrophobicity of polyethylene compared to stainless steel can be an explanation to the higher counts of adhered *Salmonella* cells on polyethylene as observed in our results.

Through the scanning electron microscopy, some irregularities in the stainless steel topography could be observed. Using the same method of microscopy, Barnes *et al.* (1) compared *Staphylococcus aureus* adherence to polished stainless steel and to rough stainless steel and observed a higher number of *S. aureus* adhered to the rough surface. As for adherence of *S.* Enteritidis to irregular surfaces, Oliveira *et al.* (24) suggested that the deepness of the irregularities were more relevant than the distance between them. Our results indicated that polyethylene presented a more irregular surface and deeper irregularities than the stainless steel, explaining the higher counts of *Salmonella* adhered to polyethylene.

In the past few years, a considerable progress has been made in order to understand the response of different bacteria to biocides (18). The evaluation of the resistance of microorganisms such as *Salmonella* to sanitizers is very important, once the correct use of such compounds can avoid foodborne outbreaks. The experiments carried out with PAA proved that the three serovars of *Salmonella* were not completely inactivated by the disinfection process, even when the concentration prescribed by the manufacturer (1500 mg kg^{-1}) is used. After disinfection with PAA, at least 1 log cm^{-2} of the serovars remained on the stainless steel surfaces. On polyethylene, 2 log cm^{-2} of *S.* Enteritidis and 3 log cm^{-2} of *S.* Bredeney were still viable. When the resistance of adhered S*almonella* to the tested surfaces is compared, a smaller decimal reduction was noticed in polyethylene after the disinfection process. This may be related to a greater irregularity of this surface. Rossoni and Gaylarde (26) evaluated the PAA efficacy in the inactivation of *Escherichia coli*, *Pseudomonas fluorescens* and *Staphylococcus aureus* adhered to stainless steel, after one hour contact with the cultures. They reported 1 log reduction of *E. coli* and *P. fluorescens* using a concentration of 250 mg kg^{-1} PAA. With a concentration of 1000 mg kg^{-1}, *S. aureus* populations were reduced in 1 log, whereas the population of *E. coli* was reduced in 2 log after 10 minutes in contact with PAA. Those authors reported that a higher decimal reduction was observed when the concentration used was increased, confirming the data obtained in the present work.

NaOCl is a disinfectant widely used in Brazil due to its low cost and broad spectrum. The FDA (Food and Drug Administration) permits its use as a biocide agent for food contact surfaces in concentrations above 200 mg kg^{-1}. When the *Salmonella* were adhered to stainless steel, a 4 log cm^{-2} reduction was achieved for *S.* Enteritidis and *S.* Typhimurium after treatment with NaOCl, whereas lower reductions were observed on polyethylene. Higher reductions rates were described by Joseph *et al.* (12), who analyzed the sensitivity of biofilms formed by *Salmonella* sp. to biocides. On a polyethylene sample treated with 100 mg kg^{-1} chlorine, 7 log cfu cm^{-2} of *Salmonella* were completely inactivated after 20 min in contact with the sanitizer. For stainless steel, 15 min were sufficient to inactivate 5 log cfu cm^{-2}. Rossoni and Gaylarde (26) evaluated the effect of 100 and 200 mg kg^{-1} NaOCl on bacterial cultures adhered to stainless steel, for a period of 10 min. The number of *E. coli* cells adhered to stainless steel was reduced from 5 to 3 log cfu cm^{-2}. Another important observation is the fact that there were no significant differences between 100 and 200 mg kg^{-1} of NaOCl when the cells were adhered to stainless steel. Similar results were presented in our study, because the reduction rates of *S.* Enteritidis and *S.* Bredeney exposed to 120 and 200 mg kg^{-1} were similar. It is important to note that these NaOCl concentrations are the ones generally recommended in Brazil for disinfection of food contact surfaces and cleaning cloths used in food preparation. Based on our results, more than 3 log cfu cm^{-2} of the *Salmonella* serovar tested remained viable after disinfection of the surfaces. In addition, -smaller reduction rates were observed for *S.* Enteritidis SE86, a strain frequently involved in salmonellosis is RS.

Sinde and Carbalo (27) observed that Quat was very efficient against *Salmonella* sp., but the bacterial reduction was also dependent on the properties of the material studied. In our work, all serovars showed a high resistance to Quat specially when adhered to polyethylene. It was mainly noted for *S.* Enteritidis SE86, once the population was almost not affected when adhered to polyethylene disinfected by different concentrations of Quat. Borowsky *et al.* (3) evaluated the resistance of *S.* Typhimurium isolated from swine to Quat. The test was made in suspension with no addition of organic matter and the levels of Quat used were 0.3 and 0.6 mg l^{-1} (15g /100 ml of active compound). According to those authors, *S.* Typhimurium samples were resistant to both concentrations after 5 min of exposure, but no resistant samples were found after 15 min.

In conclusion, the three serovars of *Salmonella* presented an important capacity of adhesion to stainless steel and to polyethylene, even in a short period of time. The sanitizers PAA, NaOCl and Quat did not inactivate all the cells adhered

to both materials, and polyethylene surfaces presented to be more difficult to disinfect than stainless steel. In some of the conditions evaluated, *S*. Enteritidis SE86 was more resistant than the other strains tested, especially to NaOCl and Quat. This resistance could explain the frequent involvement of *S*. Enteritidis SE86 in a great number of foodborne salmonellosis occurred in Southern Brazil.

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